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Lipid peroxidation in human proteinuric disease

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Background. While metabolically generated oxidants are produced locally in experimental glomerular diseases, little is still known of their significance and the respective scavenger systems in human glomerular diseases.

Methods. Here we studied kidneys from patients with congenital nephrotic syndrome of the Finnish type (CNF), a human model disease of isolated proteinuria. Expression of specific mRNAs for a major antioxidant system against lipoperoxidation [phospholipid hydroperoxide glutathione peroxidase (PHGPx)] and for mitochondrial proteins were studied in Northern blotting together with analysis of PHGPx in semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). The respective proteins and lipoperoxide (LPO) adducts malonyldialdehyde (MDA) and 4-hydroxynonenal (4-HNE) were analyzed in immunohistochemistry.

Results. PHGPx and the mitochondrially encoded subunits of cytochrome-c-oxidase were distinctly down-regulated within the glomeruli of CNF kidneys. These changes were confirmed in semiquantitative RT-PCR. Increases of lipoperoxidation products MDA and 4-HNE were constantly found in the glomeruli of CNF. In agreement with findings in CNF, similar results were obtained in biopsies from other human glomerular diseases.

Conclusions. These findings suggest that local mitochondrial damage initiates LPO, which then causes deposition of the cytotoxic LPO products in glomeruli, as seen especially in CNF kidneys. Together with down-regulation of the local antioxidant protection, these may be important pathophysiologic mechanisms in human glomerular disease.

Proteinuria is an unspecific symptom of kidney damage associated with glomerular disease regardless of the etiology. The extent of proteinuria correlates with the morphologic severity of glomerular damage, and persistent proteinuria can lead to irreversible renal scarring and loss of function ultimately requiring renal replacement therapy [1–3]. In spite of progress in establishing the inflammatory, toxic, metabolic, and genetic pathways involved, as well as identification of a variety of new glomerular cell-specific molecules, little is still known of the exact molecular mechanisms maintaining the glomerular filtration barrier.

We have previously studied the mechanisms of glomerular permeability in a human model disease of proteinuria, the congenital nephrotic syndrome of the Finnish type (CNF). In this autosomal recessive disease manifesting in the newborn with massive proteinuria, no symptoms are found from other organ systems [4–6]. The exact molecular mechanisms of the glomerular permeability failure in CNF remain to be determined. Männikkö et al have excluded defects of major matrix components of the glomerular basement membranes (GBMs) as well as some developmentally regulated genes in CNF [7, 8] and have recently established the gene defect of this syndrome by cloning a new gene, *NPHS1*, responsible for this syndrome [9]. The functions of *NPHS1* and its protein product nephrin in the kidney as well as in other tissues are not yet known. However, we [10] and others [11] have shown that nephrin localizes at the filtration slit area of podocytes.

Although metabolically generated oxidants, including reactive oxygen species (ROS) and lipoperoxides (LPOs), can be found as an epiphenomenon in many diseases [12–14], there is plenty of recent evidence that especially ROS are distinctly associated with various types of experimental glomerulonephritis [15–19]. Lipid peroxidation, mainly perturbing membrane and intracellular lipids, was recently introduced as a major pathogenetic mechanism in Heymann nephritis, the experimental model of membranous glomerulonephritis [20]. Lipid peroxidation products were found under experimental conditions in glomeruli especially at the GBM. In Western blotting of the extracellular matrix, LPO adducts were shown to bind in vitro and supposedly damage primarily type IV collagen in the rat kidney [21]. Inhibition of lipid peroxi-

Key words: nephrotic syndrome of the Finnish type, lipoperoxides, 4-hydroxynoneal, malonyldialdehyde, phospholipid hydroperoxide glutathione peroxidase, glomerular disease.

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dation with probucol, a lipophilic chain-breaking antioxidant, resulted in 80% reduction of proteinuria in Heymann nephritis [20, 21]. Together, these results strongly suggest a pathogenic role for lipid peroxidation in proteinuria, but the exact mechanisms involved are yet to be defined.

This study examined the role of lipid peroxidation in the kidneys of patients with congenital nephrotic syndrome as a human model disease of proteinuria. For this purpose, we analyzed some key markers and enzymes involved in lipid peroxidation by Northern blotting, semiquantitative reverse transcription-polymerase chain reaction (RT-PCR), and immunohistochemistry. Biopsies from other human glomerular diseases were studied in parallel.

The results show a distinct increase of local lipid peroxidation products in all of the tissue samples studied, as reflected by the increase in the lipid peroxidation products 4-hydroxynonenal (4-HNE) and malonyldialdehyde (MDA), especially in the glomerular epithelial cells. Simultaneously, the local defense against lipid peroxidation provided by the enzyme phospholipid hydroperoxide glutathione peroxidase (PHGPx) was seen to decrease, as shown by immunohistochemistry and semiquantitative RT-PCR. We propose that a local mitochondrial damage also verified here, followed by an increase in tissue damaging lipoperoxidation and failure to protect cells effectively against lipoperoxidation may be important pathophysiologic features of human proteinuric disease.

METHODS

Normal and nephrotic human kidneys

Renal tissues from CNF patients were obtained at nephrectomies performed according to an established treatment protocol. This includes daily albumin infusions, aggressive parenteral nutrition, and as the weight of 8 to 9 kg is achieved (at an age of 6 to 12 months), bilateral nephrectomy [5]. Further treatment included peritoneal dialysis and finally renal transplantation. Diagnosis of CNF ($N = 6$) was based on the typical clinical picture at birth (placental weight >40% of the weight of the newborn, edema, massive proteinuria), with exclusion of other types of congenital nephrosis, and later by the typical pathology at nephrectomy [5]. Serum albumin and protein concentrations were characteristically low, and all patients had overt proteinuria [6]. The complete laboratory findings of urine and serum of the CNF patients have been reported earlier [5, 6].

All of the procedures were approved by the ethical committee of the University of Helsinki (Helsinki, Finland).

The kidneys at nephrectomy were kept at 0°C, and glomeruli were isolated by the sieving method [22, 23] and immediately processed for RNA isolation [22]. Samples of cortical tissue were also processed for histochemistry and immunohistochemistry as previously described [22, 23].

Kidney biopsy samples, diagnosed as membranous glomerulonephritis ($N = 6$) and diabetic nephropathy (age of patients >18 years, $N = 6$), were obtained for routine diagnostic procedures. Both groups showed proteinuria exceeding 6 g/24 hours (6.2 to 15.6 g/24 hours) The biopsies were snap frozen and stored in liquid nitrogen until used.

For normal controls, cadaver kidneys ($N = 2$, ages of donors 12 and 18 years) unsuitable for transplantation because of vascular anatomical reasons (Department of Surgery, University of Helsinki) were used and, for the younger donors ($N = 2$, ages 3 and 5 years) a normal pole of a kidney removed because of Wilms' tumor was used. These were processed as described previously in this article for CNF, with an exception that the cadaver kidneys were perfused with Ringer's buffer solution via the renal artery.

Immunofluorescence microscopy

To study the presence and distribution of lipid peroxidation products 4-HNE, MDA as well as the PHGPx, superoxide dismutase and catalase, frozen sections of normals and CNF kidney, and kidney biopsy samples of patients from membranous glomerulonephritis and diabetic nephropathy were cut at 3 to 4 μm, fixed in acetone at −20°C for five minutes, and washed in phosphate-buffered saline (PBS). Thereafter, polyclonal antibodies against 4-HNE, MDA [24], PHGPx [25] (Cu/Zn) superoxide dismutase (Serotec, Oxford, UK), and catalase (Serotec) were flooded over sections for 30 minutes. After washing, the tissue sections were further incubated with rat anti-rabbit IgG (Boehringer, Mannheim, Germany) coupled with fluorescein isothiocyanate (FITC). An Olympus OX50 microscope equipped with an epi-illuminator and a filter system for FITC fluorescence was used for microscopy.

RNA isolation

Total glomerular RNA was isolated using the guanidinium thiocyanate-cesium chloride method from the CNF and control kidneys as described earlier [26].

Northern blotting

Total RNA (30 μg RNA/lane) from CNF and normal human cortex samples was electrophoresed and transferred to nylon membranes. Membranes were ultraviolet cross-linked (Stratalinker; Stratagene, La Jolla, CA, USA) and hybridized as described earlier [22]. To control the total RNA content and lack of degradation in the analyzed preparations, the blots were rehybridized with a human β-actin probe. For autoradiography, the

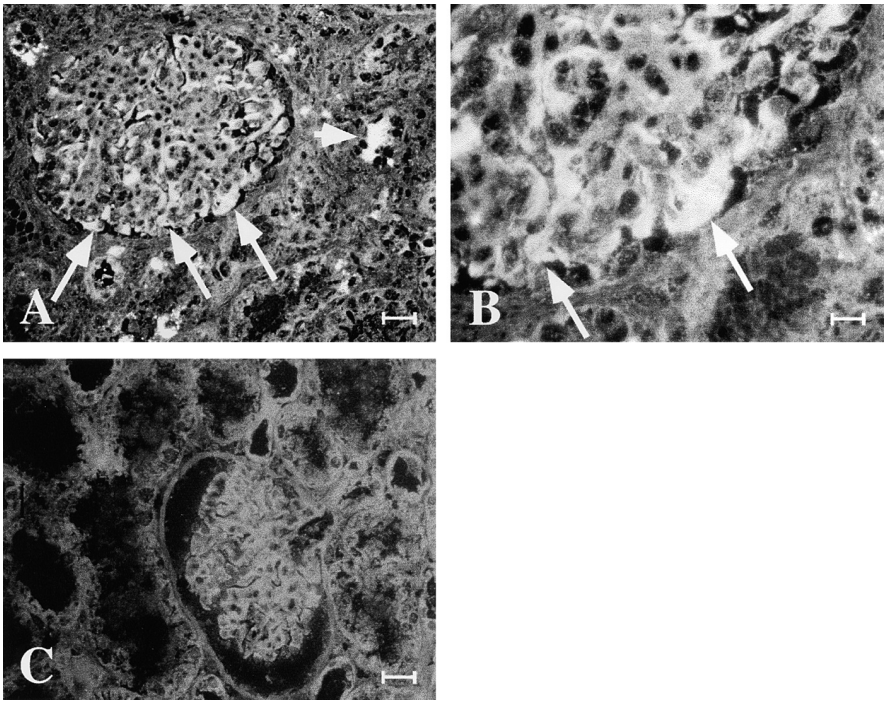

Fig. 1. Immunostaining of congenital nephrotic syndrome of the Finnish type (CNF) patient kidneys (*A* and *B*) and normal human kidney cortex (*C*) with anti–4-hydroxynonenal (anti-4-HNE) antibodies. Strong, patchy reactivity at areas facing the urinary space in glomeruli is obvious in CNF kidneys (A and B, arrows), whereas faint background reactivity is seen in normal kidney (C). Bar 10 μm (A and C) and 3 μm (B).

filters were exposed on Fuji Bas IIIS Imaging Plates, and the expression was quantitated using a phosphoimager and accompanying MacBAS software (Fuji Photo Film Co., Tokyo, Japan).

To detect expression of genes for the mitochondrial respiratory chain complex IV subunits, cytochrome-c-oxidase (COX I, COX IV) as markers for mitochondrial function and the main protective enzyme (PHGPx), we used specific cDNA probes in Northern analysis. Probe for PHGPx was produced by RT-PCR using random hexamer primers in reverse transcription, and PHGPx-specific primers were used in PCR. Specific primers were based on the mRNA sequence of human PHGPx (Genbank accession number X71973). For 5′ primer (nt 220 to 238), we used 5′-AGG ACA TCG ACG GGC ACA T and for 3′ primer (nt 642 to 661) 5′-GGC AGG TCC TTC TCT ATC AC. The amplified PCR fragment was cloned into the pGEM-T vector (Promega, Madison, WI, USA). The authenticity of the cloned fragment was verified by DNA sequencing (DNA sequencing kit, version 2.0; United States Biochemicals, Cleveland, OH, USA).

To reveal transcript levels of COX I, a plasmid B36 containing a 363 bp long cDNA fragment of *COX I* gene and plasmid pCOX4 containing a 682 bp COX IV cDNA fragment (courtesy of Dr. M.I. Lomax) were used.

Semiquantitative RT-PCR

We also determined the level of PHGPx transcription by semiquantitative RT-PCR from the RNA of isolated glomeruli. For this purpose, total RNA from isolated glomerular fractions was used as previously described [27]. Oligo(dT)-primer was used in reverse transcription, and oligonucleotide primers specific for PHGPx were used in PCR reactions. The semiquantitation is based on the amount of housekeeping (β-actin, GAPDH) amplification product [27].

RESULTS

Lipid peroxidation products

Immunohistochemistry of CNF cortical kidney samples revealed a consistent deposition of both 4-HNE (Fig. 1 A, B) and MDA (Fig. 2 a, b) within glomeruli, whereas only traces of glomerular reactivity could be seen within control kidneys (Figs. 1C and 2e). 4-HNE and MDA were seen as finely granular dense reactivity at the glomerular basement membranes and preferentially within podocytes, as determined by their localization next to the urinary space (Figs. 1B and 2b). Similarly, in biopsy samples of human membranous ($N = 6$) and minimal change ($N = 6$) patients, 4-HNE and MDA (Fig. 2 c, d) were seen within glomeruli, particularly at the GBM and mesangium.

PHGPx immunohistochemistry

In line with previous studies, a heavy, finely granular staining for the lipoperoxidation protective enzyme PHGPx was observed in immunohistochemistry within podocytes in the control kidney (Fig. 3a). In all CNF

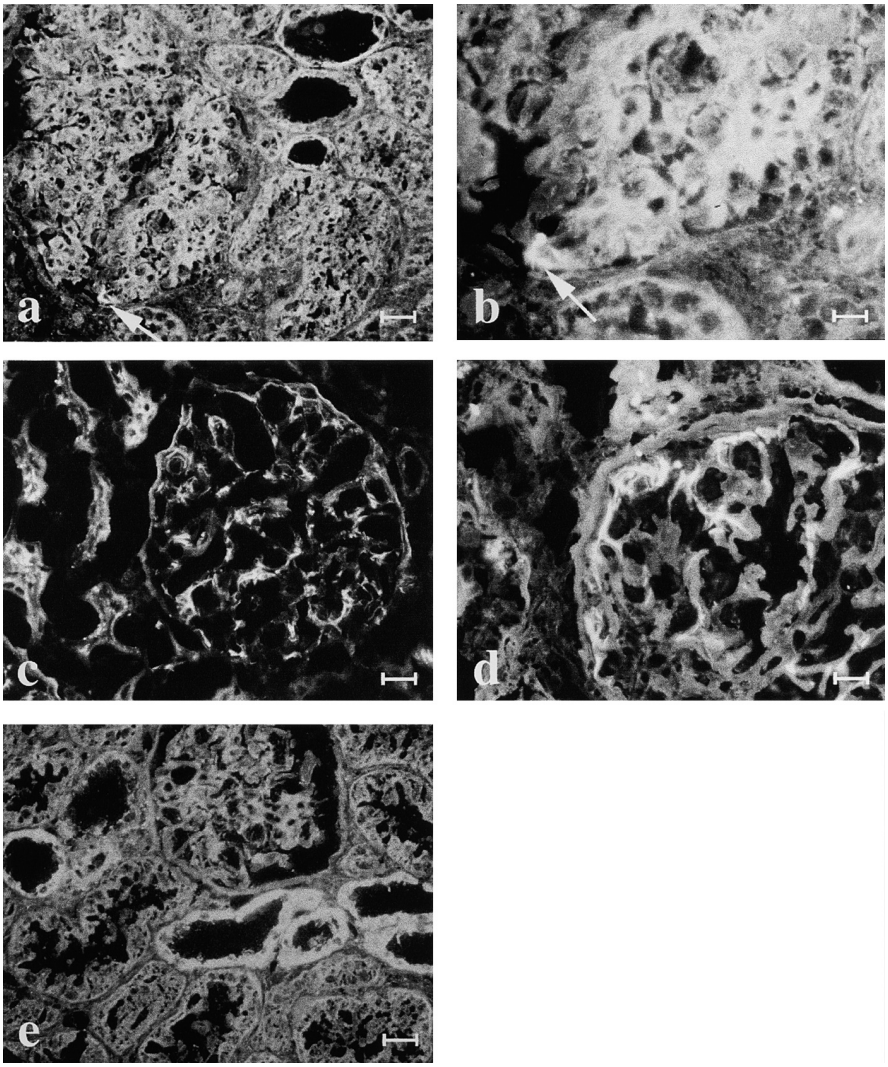

Fig. 2. Immunostaining of CNF patient kidneys (*a* and *b*), biopsy sample of membranous glomerulonephritis (*c*), diabetic nephropathy (*d*), and normal human kidney (*e*) with anti-MDA antibodies. Reactivity is seen in apparent podocytes (arrows in a and b) but also in the mesangium and at the basement membranes (b–d). (Bar = 10 μm for a, c, and e; and 3 μm for b and d).

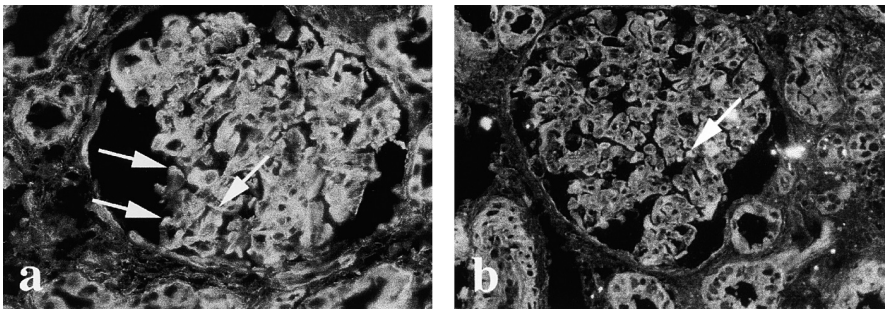

Fig. 3. Reactivity of anti-phospholipid hydroperoxide glutathione peroxidase (anti-PHGPx) antibodies in normal kidney cortex (*a*) and in CNF kidney (*b*). Note the prominent reactivity in a podocyte-like pattern in (a, arrows) with a decreased reactivity pattern in CNF (b). Magnification ×260.

samples, glomerular reactivity of anti-PHGPx antibodies was decreased (Fig. 3b). No difference between the reactivity of subcapsular and juxtamedullary glomeruli could be observed. In contrast to CNF samples, no major decrease in staining intensity of the patient biopsy samples was seen. In agreement with previous results, no specific glomerular reactivity for the predominant antioxidant enzymes superoxide dismutase and catalase could be seen within glomeruli of control or patient kidney biopsies (data not shown).

Northern blotting

To detect changes in the RNA levels of PHGPx and, as markers for mitochondrial function, COX subunits

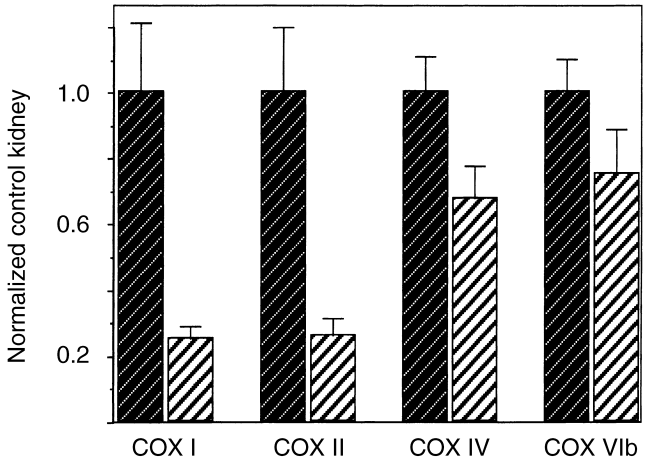

Fig. 4. RNA expression levels of kidney cortical cytochrome-c-oxidase (COX) subunits encoded by the mitochondrial (COX I, COX II) and nuclear genome (COX IV, COX VIb) in control human kidney (NHK; ▩) and CNF (▨). When the values of the control kidneys are normalized to 1.0, the values of the mitochondrially encoded components are decreased to 25.9 and 27.3% of the control ($P < 0.001$ for both COX I and COX II vs. controls), whereas no significant change in the nuclearly encoded components is seen.

Table 1. Transcript abundance in isolated glomeruli as determined by RT-PCR and analyzed by densitometric scanning

	COX I	Cyt b	COX 7	PHGPx
CNF	0.75	1.65	1.14	0.92
NHG	4.02	2.66	1.59	1.41
CNF/NHG	0.19	0.62	0.72	0.65

Signals are expressed relative to the level of β-actin expression and normalized to the mean of the CNF samples (Fig. 5). Both for control and CNF samples, $N = 3$. Abbreviations are in the **Appendix**.

encoded by the mitochondrial (COX I) and nuclear genome (COX IV), Northern blotting analysis of total RNA from cortical kidney was performed. The results showed down-regulation of the mitochondrially encoded COX I and COX II to 25 and 26% of the controls ($P < 0.001$ for both), whereas the nuclearly encoded subunits COX IV and COX VIIa were at 68 and 75% of controls, respectively ($P = 0.21$ and 0.68, respectively; no statistical significance; Fig. 4). No significant differences in PHGPx from the RNA isolated from total kidney cortical tissue of CNF and normal kidneys were observed.

RT-PCR

Northern analysis of total cortical kidney failed to reflect the mRNA abundance in glomeruli correctly as it composed only approximately 5% of the total cortical volume. Thus, to verify the glomerular expression level of PHGPx message, a semiquantitative RT-PCR analysis was used (Table 1 and Fig. 5). In agreement with protein expression data in immunohistochemistry (Fig. 3), a similar down-regulation of the PHGPx-specific mRNA level was suggested (68% of the controls, $P = 0.24$; no statistical significance).

DISCUSSION

Our study shows that a decrease of the mitochondrial enzyme COX and a concomitant increase of tissue damaging lipid peroxidation are associated with human glomerular disease. Furthermore, we show for the first time a reciprocal local decrease of PHGPx, the major enzyme system protecting against lipid peroxidation in glomeruli [25, 28]. Together, our results suggest a causal relationship between mitochondrial damage, LPO production, and proteinuria.

The defective gene in CNF kidneys has recently been identified [9]. This gene codes for a new transmembrane protein, nephrin, with immunoglobulin-like sequences of cell adhesion molecules [9]. There is no direct evidence as yet that nephrin is involved in regulating the production of ROS. However, the genes, for example, for PHGPx as well as COX 6A, COX 7, NADH-hydrogenase, and mitoribosomal proteins, locate in close proximity of the nephrin gene in chromosome 19q13.1, possibly indicating a cluster of genes in regulating mitochondrial functions. It is noteworthy that Mandel, Bacallao, and Zampighi have shown that epithelial permeability is critically dependent on available adenosine 5′-triphosphate (ATP) energy in vitro [29]. Disruption of cellular ATP levels rapidly perturbs the actin network important for maintaining specialized cellular architecture [30–32]. Thus, the common morphologic findings of proteinuria, the flattening and retraction of podocyte foot processes may be due to remodeling of the actin skeleton in response to a decrease in ATP energy in these cells. Interestingly, our recent results have clearly shown a strong decrease of all mitochondrial functions, including ATP formation in CNF glomeruli (Solin et al, manuscript in preparation) [33].

The other human proteinuric diseases studied, adult minimal change disease of unknown etiology and membranotic nephropathy, showed similar accumulation of the LPO adducts and a down-regulation of PHGPx within glomeruli, which is suggestive of a general phenomenon shared with other noninflammatory human proteinuric diseases as they are in the experimental models.

Superoxide and hydroxyl radicals and hydrogen peroxide are the highly ROS mainly produced in mitochondria as unavoidable by-products of aerobic respiration [34]. In primary and secondary mitochondrial diseases, an increase in ROS production is commonly seen that subsequently leads to a decrease of energy production [34]. Luo et al have recently shown that this also causes an increase in hydrogen peroxide levels and lipid peroxidation products in human mitochondrial disease with

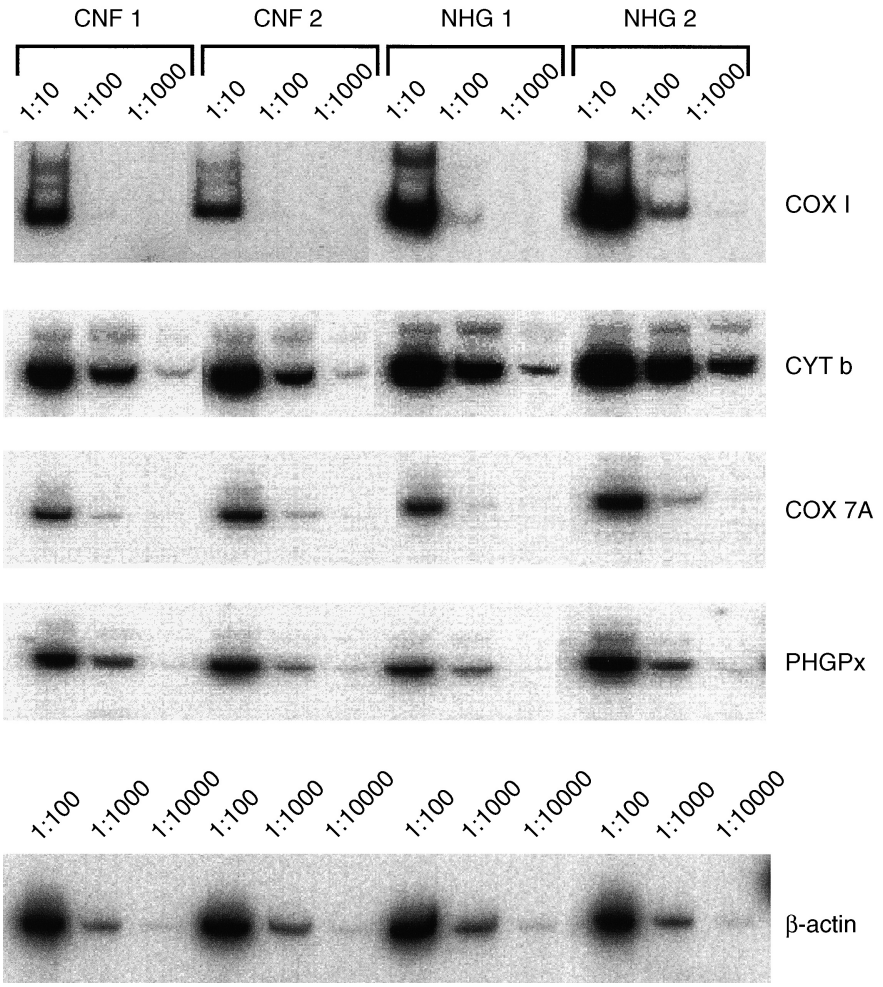

Fig. 5. Semiquantitative RT-PCR of CNF and normal human kidney glomeruli (NHG 1 and NHG 2) with transcripts of mitochondrially encoded cytochromes I (COX I) and cytochrome b (CYT b), nuclearly encoded cytochrome 7A (COX 7A) and phospholipid hydroperoxide glutathione peroxidase (PHGPx). cDNAs were serially diluted to the range of linear amplification (10^{-1} to 10^{-4}); β-actin expression was used to obtain comparable cDNA concentrations in all samples. Note the down-regulation of COX I, CYT b, and PHGPx.

complex I deficiency [35]. Thus, regardless of the primary damage to mitochondria locally, lipoperoxidation appears to be an important pathophysiologic effector mechanism involved. However, to date little is known of the role of mitochondrial functions and dysfunction in renal disease. Indirect evidence suggests that glomerular permeability in particular is highly dependent on the maintenance of energy balance [36]. It is also interesting that local LPO scavenger enzymes appear particularly important for glomeruli, as evidenced by the abundant PHGPx found in podocytes but not in other parts of the nephron. In fact, the only other major site of PHGPx expression is in the testis [25]. Further studies are needed to elucidate the precise role of lipoperoxidation end-products for human glomerular disease.

In this study, we could show distinct deposition of MDA and 4-HNE adducts in glomeruli in human proteinuria similar to that in Heymann nephritis [12], where they appear to directly perturb the functional GBM. This is in good agreement with our earlier results showing splitting of GBM nidogen in the kidneys of patients with congenital nephrotic syndrome [37]. In line with other results of this study, a decrease of the protective PHGPx could be shown by immunohistochemistry in the glomerulus, where the changes appeared reciprocal to the increase in the lipoperoxidation end-products MDA and 4-HNE. In experimental models of proteinuria our recent results show that mitochondrial damage, MDA and 4-HNE accumulation, as well as decrease of PHGPx are found prior to the onset of proteinuria (Holthöfer et al, manuscript submitted for publication). Together, these results suggest a strong causal relationship of mitochondrial damage and proteinuria.

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APPENDIX

Abbreviations used in this article are: CNF, congenital nephrotic syndrome of the Finnish type; COX, cytochrome-c-oxidase; FITC, fluorescein isothiocyanate; GBM, glomerular basement membrane; 4-HNE, 4-hydroxynonenal; LPO, lipoperoxide or lipoperoxidation; MDA, malonyldialdehyde; PHGPx, phospholipid hydroperoxide glutathione peroxidase; ROS, reactive oxygen species; and RT-PCR, reverse transcription-polymerase chain reaction.

REFERENCES

1. Kerjaschki D: Dysfunctions of cell biological mechanisms of visceral epithelial cells (podocytes) in glomerular diseases. *Kidney Int* 45:300–303, 1994
2. Kerjaschki D, Neale TJ: Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis). *J Am Soc Nephrol* 7:2518–2526, 1996
3. Rennke HG: How does glomerular epithelial cell injury contribute to progressive glomerular damage. *Kidney Int* 45:S58–S63, 1994
4. Hallman N, Norio R, Kouvalainen K: Main features of the congenital nephrotic syndrome. *Acta Pediatr Fenn* 172:75–78, 1967
5. Holmberg C, Antikainen M, Rönnholm K, *et al:* Management of congenital nephrotic syndrome of the Finnish type. *Pediatr Nephrol* 9:87–93, 1995
6. Laine J, Jalanko H, Holthöfer H, *et al:* Post-transplantation nephrosis in congenital nephrotic syndrome of the Finnish type. *Kidney Int* 44:867–874, 1993
7. Männikkö M, Lenkkeri U, Kashtan CE, *et al:* Haplotype analysis of congenital nephrotic syndrome of the Finnish type in Finnish and non-Finnish families. *J Am Soc Nephrol* 7:2700–2703, 1996
8. Kestilä M, Männikkö M, Holmberg C, *et al:* Exclusion of eight genes as mutated loci in congenital nephrotic syndrome of the Finnish type. *Kidney Int* 45:986–990, 1994
9. Kestilä M, Lenkkeri U, Männikkö M, *et al:* Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1:575–582, 1998
10. Ahola H, Wang SX, Luimula P, *et al:* Cloning and expression of the rat nephrin homolog. *Am J Pathol* 155:907–913, 1999
11. Ruotsalainen V, Ljungberg P, Wartiovaara J, *et al:* Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci USA* 96:7962–7967, 1999
12. Halliwell B: Free radicals, antioxidants and human disease: Curiosity, cause or consequence? *Lancet* 344:721–724, 1994
13. Janssen YMW, van Houten B, Borm PJA, *et al:* Cell and tissue responses to oxidative damage. *Lab Invest* 69:261–274, 1993
14. Johns DR: Mitochondrial DNA and disease. *N Engl J Med* 333:638–644, 1995
15. Diamond JR: The role of reactive oxygen species in animal models of glomerular disease. *Am J Kidney Dis* 19:292–300, 1992
16. Johnson RJ, Lovett D, Lehrer RI, *et al:* Role of oxidants and proteases in glomerular injury. *Kidney Int* 45:352–359, 1994
17. Neale TJ, Ullrich R, Ojha P, *et al:* Reactive oxygen species and neutrophil respiratory burst cytochrome b558 are produced by kidney glomerular cells in passive Heymann nephritis. *Proc Natl Acad Sci USA* 90:3645–3649, 1993
18. Shah SV: Oxidant mechanisms in glomerulonephritis. *Semin Nephrol* 11:320–326, 1991
19. Thakur V, Walker PD, Shah SV: Evidence suggesting a role for hydroxyl radical in puromycin aminonucleoside-induced proteinuria. *Kidney Int* 34:494–499, 1988
20. Neale TJ, Ojha PP, Exner M, *et al:* Proteinuria in passive Heymann nephritis is associated with lipid peroxidation and formation of adducts on type IV collagen. *J Clin Invest* 94:1577–1584, 1994
21. Riedle B, Kerjaschki D: Reactive oxygen species cause direct damage of Engelbreth-Holm-Swarm matrix. *Am J Pathol* 151:215–230, 1997
22. Haltia A, Solin ML, Jalanko H, *et al:* Mechanisms of proteinuria: Vascular permeability factor in congenital nephrotic syndrome of the Finnish type. *Pediatr Res* 40:652–657, 1996
23. Holthofer H, Reivinen J, Solin ML, *et al:* Decrease of glomerular disialogangliosides in puromycin nephrosis of the rat. *Am J Pathol* 149:1009–1015, 1996
24. Montine TJ, Amarnath V, Martin ME, *et al:* E-4-hydroxy-2-nonenal is cytotoxic and cross-links cytoskeletal proteins in P19 neuroglial cultures. *Am J Pathol* 148:89–93, 1996
25. Conz PA, Bevilacqua PA, LaGreca G, *et al:* Phospholipid hydroperoxide glutathione peroxidase in the normal human kidney: A possible role in protecting cell membranes. *Exp Nephrol* 1:376–378, 1993
26. Chirgwin JM, Przybyla AC, MacDonald RJ, *et al:* Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294–5295, 1979
27. Kretzler M, Fan G, Rose D, *et al:* Novel mouse embryonic renal marker gene products differentially expressed during kidney development. *Am J Physiol* 271:770–777, 1996
28. Ursini F, Maiorino M, Brigelius-Flohe R, *et al:* Diversity of glutathione peroxidases. *Methods Enzymol* 252:38–53, 1995
29. Mandel LJ, Bacallao R, Zampighi G: Uncoupling of the molecular fence and paracellular gate functions in epithelial tight junctions. *Nature* 361:552–555, 1993
30. Gabai VL, Kabakov AE: Rise in heat-shock protein level confers tolerance to energy deprivation. *FEBS Lett* 27:247–250, 1993
31. Mundel P, Kriz W: Structure and function of podocytes: An update. *Anat Embryol* 192:385–397, 1995
32. Shirato I, Sakai T, Kimura K, *et al:* Cytoskeletal changes in podocytes associated with foot process effacement in Masugi nephritis. *Am J Pathol* 148:1283–1289, 1996
33. Holthöfer H, Kretzler M, Haltia A, *et al:* Altered gene expression and functions of mitochondria in human nephrotic syndrome. *FASEB J* 13:523–532, 1999
34. McCormack JG, Denton RM: Mammalian mitochondrial metabolism and its regulation, in *Mitochondria: DNA, Proteins and Diseases*, edited by Darley-Usmar V, Schapira AHV, London, Portland Press, 1994, pp 221–239
35. Luo X, Pitkänen S, Kassovska-Bratinova S, *et al:* Excessive formation of hydroxyl radicals and aldehyde lipid peroxidation products in cultured skin fibroblasts from patients with complex I deficiency. *J Clin Invest* 99:2877–2882, 1997
36. Kerjaschki D: Polycation-induced dislocation of slit diaphragms and formation of cell junctions in rat kidney glomeruli. *Lab Invest* 39:430–440, 1978
37. Ljungberg P, Haltia A, Kuusela P, *et al:* Noncollagenous matrix components of glomeruli in congenital nephrotic syndrome of the Finnish type: Evidence of abnormal splitting of nidogen. *Exp Nephrol* 4:286–294, 1996